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IN RATS OF DIFFERENT SEX AND AGE DURING
MUSCULAR LOADS AND HYPOKINESIA

V. D. Rozanova, G. A. Antonova

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ACTIVITY OF CHOLINESTERASES OF BLOOD AND HEART IN RATS OF
DIFFERENT SEX AND AGE DURING MUSCULAR LOADS AND HYPOKINESIA

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V. D. Rozanova and G. A. Antonova

Laboratory for Age-related and Comparative Pathophysiology
(Chief: I. A. Arshavskiy)

Institute of General Pathology and Pathophysiology, AMS USSR
Moscow

The activity of acetylcholinesterase (Ache) and butyrylcholinesterase (Bche) in the blood and the sections of the heart in white male rats 3 and 13 months old was lower than in females. In old animals (25 months) difference according to sex in Ache and Bche activity in the sections of the heart is absent. In males and females 3 and 13 months developing under conditions of muscle loads, Ache and Bche activity in the blood and all sections of the heart was lower, while in those developing under conditions of hypokinesia it was higher than in control rats. In rats of all age groups, regardless of age, sex, or conditions of development, a decrease in Ache and Bche activity was found from the sinoatrial node to the heart apex.

Key words: acetylcholinesterase, butyrylcholinesterase, hypokinesia, muscle load.

Laboratory work has shown the significance of the characteristics of skeletal-muscle development as a factor in determining the level of energy expenditure and activity of the

* Numbers in margin indicate pagination of foreign text.

vegetative organs in the ontogenesis process, which has found expression in the "energy principle of skeletal muscles," formulated by I. A. Arshavskiy.²⁻⁴ The development of the organism under conditions of systematic muscle load leads to the economizing of vegetative functions, linked to the strengthening of cholinesterase mechanisms of regulation⁷⁻⁹. In connection with the fact that in previous works study was made of changes in activity in, for the most part, true cholinesterase without differentiation according to sex¹ and only in the blood⁹, in the present work acetylcholinesterase (Ache) and butyrylcholinesterase (Bche) activity was studied not only in the blood, but also in the tissues of various sections of the heart in rats (male and female) which developed under varying levels of muscle load. In addition, the task was assumed of investigating the changes in acetylcholinesterase and butyrylcholinesterase activity according to sexual difference in control males and females during the period from sexual maturity to old age.

PROCEDURE

Studies were carried out on mongrel rats, male and female. Animals in groups 1 and 2 (males and females) developed from 1 to 3 months under load conditions from running on a treadmill on alternate days with a gradual increase in running time from 5 to 30 minutes. The third group (males) developed from 1 to 3 months under conditions of hypokinesia, created by being kept in narrow cages. Animals in group 4 (males) developed from 1 to 13 months of age under load conditions from

running on alternate days from 30 to 100 minutes. Rats in the first three groups were sacrificed at age 3 months and the rats in group 4 at 13 months. At these same times, rats in the three control groups of corresponding age and sex, which developed under normal conditions, being kept in a vivarium, were also sacrificed. In addition, in connection with the second task, investigating the changes in acetylcholinesterase and butyrylcholinesterase up to the period of aging, 3 additional groups of control rats were taken for study: females 13 months old, males and females 25 months old. From each rat, blood was taken, as well as tissues from three sections of the heart: regions of the sinoatrial and atrioventricular nodes and the contractile myocardium of the apex of the left /1000 ventricle. Ache and Bche activity was determined by the method of A. A. Pokrovskiy⁶ in blood plasma and in tissue extracts from the heart sections and was expressed in micromoles per gram or per ml per minute. The substrate for determining Ache activity was acetylcholinyloride and for Bche, butyrylcholiniodide. The data were processed statistically according to Student.

RESULTS OF THE INVESTIGATION AND DISCUSSION

In comparing the data from the control animals 3, 13, and 25 months old it is clear that the activity of the enzymes under study in the blood is lower in males than in females: Ache by 60, 39, and 32% and Bche by 78, 49, and 63%

respectively (see table). Comparison of the enzyme activity values from the sections of the heart also shows that in males of 3 and 13 months they are significantly lower than in females. In old rats (25 months) the sex-related differences in activity values for Ache and Bche in the sections of the heart are leveled out or are absent. In the blood these differences do not disappear, but for Bche they even increase in comparison with 13 month olds.

Development of animals under conditions of systematic muscle loads induces a significant decrease in Ache and Bche activity in males and females as compared to the values in control rats of corresponding age and sex. In 3 month old males this decrease, in the majority of tests, is much sharper than in females of the same age. Ache activity in the blood of male test animals was lowered, compared to control males, by 33%, while in test females the figure was 25%. The degree of decrease in Ache and Bche activity in test males in the atrioventricular node (by 65 and 69%) and Bche activity in the myocardium (by 70%) compared to the decrease in test females (by 46, 38, and 35%) is also quite sharp. In the remaining cases no substantial differences were found in the degree of decrease in Ache and Bche activity in test males and females as compared to control animals. With regard to the decrease in the absolute values of all the indices under study in test males of 3 and 13 months, they were in all cases significantly lower than in test females.

The development of males from 1 to 13 months under conditions of hypokinesia leads to the opposite changes in the activity of the enzymes under investigation. In them a significant increase is noted in Ache and Bche activity in the blood and in almost all sections of the heart (by 61-91%) compared to 3 month old control males. In the sinoatrial and atrioventricular nodes the increase in Ache activity under hypokinesia was markedly less (by 46 and 31%).

Examination of the dynamics of the changes in Ache and Bche in the sections of the heart allows the determination of a decrease in the activity of these enzymes from the sinoatrial node to the atrioventricular node and to the apex of the heart. This decrease of 40-58% in the majority of cases takes place in all control and test rats regardless of age, sex, and level of muscle activity. Only in 3 month old control females is the gradient of enzyme activity less pronounced.

It should also be observed that in the blood Ache activity is higher than Bche activity, while in the heart tissues, on the other hand, Bche activity is higher than or does not differ from Ache activity.

The sharp differences according to sex, revealed in the study, of the activity level of enzymes that break down acetylcholine in control rats allows us to assume a higher level of cholinergic background in regulation in males than in females. These differences are more sharply pronounced in the third month of life (the period when sexual maturity

begins) and decrease somewhat by 13 months — the end of the reproductive period, when the first symptoms of aging appear.

Previous laboratory works^{10,11} have shown that male and female rats differ in the level of development of their skeletal musculature, and the level of energy and activity in their vegetative organs. In males, the O₂ requirement and heart rate at 4 and 13 months are 13, 18, 15, and 16% lower respectively than in females^{10,11}. It may be assumed that lowered Ache and Bche activity in the blood of males /1002 provides a more pronounced economizing of energy expenditure in them at rest than in females. Lowered Ache and Bche activity in the region of the sinoauricular node in 3 and 13 month old control males coincides with lower heart rate values in these age groups (305±9.1 and 292±4.7 per minute) as compared to the females (360±5.1 and 344±3.9 per minute). Since tonus vagus is hardly pronounced in rats², it may be assumed that the lower heart rate values in males are due to lowered Ache activity in the pacemaking node of the heart. By old age (25 months), sex-related differences in Ache and Bche activity in tissues from the sections of the heart almost disappear. This may be explained by the decrease in acetylcholine synthesis in the auricle in old rats, in connection with decreased choline acetylase activity⁵. Our data on the disappearance by age 25 months of sex-related differences in Ache activity at the sinoatrial node allows us to assume the absence of differences in old rats with respect to acetylcholine content

Acetylcholinesterase and butyrylcholinesterase activity in the blood (mole/ml/min) and in the cardiac tissues (mole/g/min) in rats of various age (males and females): controls (C), those developing under conditions of muscle load (M) and hypokinesia (H) (M + m).

Age (In months and sex)	Acetylcholinesterase Activity					
	Blood			Sinoatrial Node		
	C	M	H	C	M	H
3♂	0.86 ± 0.03	0.58 ± 0.05	1.65 ± 0.01	1.54 ± 0.03	1.00 ± 0.009	2.20 ± 0.03
3♀	2.14 ± 0.01	1.60 ± 0.02	-	3.20 ± 0.02	2.10 ± 0.04	-
13♂	0.95 ± 0.01	0.82 ± 0.01	-	1.40 ± 0.04	0.50 ± 0.007	-
13♀	1.55 ± 0.01	-	-	2.65 ± 0.01	-	-
25♂	1.04 ± 0.03	-	-	1.47 ± 0.01	-	-
25♂	1.53 ± 0.01	-	-	1.49*± 0.02	-	-
	Atrioventricular Node			Myocardium of Left Ventr.		
	C	M	H	C	M	H
	C	M	H	C	M	H
3♂	1.22 ± 0.02	0.38 ± 0.04	1.60 ± 0.01	0.93 ± 0.009	0.39 ± 0.01	1.50 ± 0.03
3♀	2.60 ± 0.01	1.40 ± 0.02	-	2.60 ± 0.03	1.20 ± 0.04	-
13♂	1.00 ± 0.01	0.40 ± 0.009	-	0.66 ± 0.02	0.29 ± 0.01	-
13♀	1.40 ± 0.008	-	-	1.18 ± 0.01	-	-
25♂	0.05 ± 0.89*	-	-	0.69 ± 0.01	-	-
25♂	0.89*± 0.007	-	-	0.73*± 0.01	-	-

Age (In months and sex)	Butyrylcholinesterase Activity					
	Blood			Sinoatrial Node		
	C	M	H	C	M	H
3 ♂	0.55 ± 0.02	0.27 ± 0.03	1.82 ± 0.01	1.75 ± 0.01	1.13 ± 0.01	3.00 ± 0.009
3 ♀	2.50 ± 0.03	1.28 ± 0.01	- -	3.80 ± 0.009	2.50 ± 0.01	- -
13 ♂	0.75 ± 0.02	0.45 ± 0.01	- -	1.70 ± 0.03	1.46 ± 0.01	- -
13 ♀	1.48 ± 0.003	- -	- -	2.75 ± 0.02	- -	- -
25 ♂	0.72 ± 0.008	- -	- -	2.16 ± 0.01	- -	- -
25 ♂	1.97 ± 0.01	- -	- -	2.21 ± 0.01	- -	- -
3 ♂	Atrioventricular Node			Myocardium of Left Ventr.		
	1.40 ± 0.009	0.49 ± 0.01	2.50 ± 0.03	1.22 ± 0.04	0.36 ± 0.03	2.00 ± 0.02
3 ♀	2.90 ± 0.01	1.80 ± 0.04	- -	2.90 ± 0.01	1.90 ± 0.02	- -
13 ♂	1.18 ± 0.02	0.39 ± 0.01	- -	0.74 ± 0.03	0.29 ± 0.01	- -
13 ♀	1.46 ± 0.01	- -	- -	1.14 ± 0.01	- -	- -
25 ♂	1.37 ± 0.01	- -	- -	0.98 ± 0.05	- -	- -
25 ♂	1.43*± 0.02	- -	- -	1.23 ± 0.006	- -	- -

Note. The differences between values in males and females within each age group as well as in groups M and H are statistically reliable as compared to the corresponding control group. Unreliability of sex-related differences in groups of rats 25 months old is denoted by the asterisk *. The number of 3 month old animals in groups C and M is always 10, in group H — 7; 13 month old males in group C — 10; in all remaining groups — 5.

and choline acetylase activity.

The sharp differences according to sex that we discovered in the activity of the enzymes that break down acetylcholine allow us to conclude that studies on questions of neuro-humoral regulation in pubertal rats should be carried out with obligatory division of them according to sex.

Previous laboratory studies^{10,11} have shown that muscle loads lead by age 4 and 13 months to a decrease in basal metabolism in males by 19 and 26% and in females by 23 and 18% compared to control rats of corresponding age and sex. In test rats of both sexes, the heart rate also decreased in comparison with control animals. Comparison of data from the present work with material from previous laboratory studies allows the conclusion that the decrease in Ache activity in the blood and in cardiac tissues is associated with the economizing of energy expenditure and activity of the heart. This conclusion is substantiated by analysis of the data obtained from 3 month old males raised under conditions of hypokinesia. Increased Ache activity in them is associated with increased energy expenditure and heart rate^{10,11} as compared to control rats and especially to rats developing from the age of one month under conditions of muscle load.

A previous study¹⁰ has shown that in rats of both sexes by age 4 months, the catecholamine content in the adrenal glands and in the truncus cerebri is raised and in males this increase is even slightly greater than in females. In connection

with this, the differences by sex in energy expenditure and cardiac activity under resting conditions, as well as the decrease in these indices in rats developing under conditions of muscle loads, may basically be explained by the substantial decrease in Ache activity in the blood and in cardiac tissues.

The decrease we found in Ache and Bche activity in the direction from the sinoatrial node to the apex of the heart in rats of all control and test groups is apparently linked to the gradient of the heart's automatic activity and to the peculiarities of acetylcholine distribution^{13,14}. This gradient takes place in animals of other species and is determined, as shown¹, from the first day of life.

It should be noted that the dynamics of age-related changes in Ache and Bche activity in the blood and cardiac tissues are different in control males and females developing under normal conditions. In males from 3 to 25 months Ache and Bche activity changes insignificantly, while in females a sharp decrease is noted. In this way, the disappearance of sharp sex-related differences in Ache and Bche activity by old age occurs basically by way of the decrease among the females.

LITERATURE

1. Antonova, G. A. "Dinamika aktivnosti atsetilkholin- i butirilkholinesterazy v tkanyakh serdtsa u sobak v postnatal'nom ontogeneze" ("Dynamics of Acetylcholinesterase and butyrylcholinesterase Activity in Cardiac Tissues of Dogs in postnatal ontogenesis"). Byulleten' eksperimental'noy biologii

i meditsiny, 1973, 76, 12, 7 - 9.

2. Arshavskiy, I. A. Ocherki po vozrastnoy fiziologii ("Outline of Age-related Physiology"), Publ.: Meditsina, Moscow, 1967.

3. Arshavskiy, I. A. "Rol' energeticheskikh faktorov v kachestve vedushchikh zakonomernostey ontogeneza" ("The Role of Energy Factors as the Primary Factors in Ontogenesis"), in the book Vedushchie faktory ontogeneza ("Primary Factors of Ontogenesis"). Publ.: Naukova Dumka, Kiev, 1972, 43 - 72. /1003

4. Arshavskiy, I. A. "Fiziologicheskie mekhanizmy nekotorykh osnovnykh zakonomernostey ontogeneza" (Physiological Mechanisms of Several Basic Factors in Ontogenesis"). Uspekhi fiziologicheskikh nauk, 1971, 2, 4, 100 - 141.

5. Verkh ratskiy, N. S. "Obmen mediatorov i reaktsiya effektorov na kholinergicheskie i adrenergicheskie vliyaniya v starosti" ("Mediator Metabolism and Effector Reaction to Cholinergic and Adrenergic Influences in Old Age"). Dissertation, Kiev, 1971.

6. Pokrovskiy, A. A. Rukovodstvo po izucheniyu pitaniya i zdorov'ya naseleniya ("Handbook for the Study of the Nutrition and Health of the Populace"). Moscow, 1966, 144 - 147.

7. Rozanova, V. D. Ocherki po eksperimental'noy vozrastnoy farmakologii ("Outline of Experimental Age-Related Pharmacology"). Publ.: Meditsina, Leningrad, 1968.

8. Rozanova, V. D. "Neyro-gumoral'nye mekhanizmy regulyatsii gomeostazisa i intensivnosti rosta v razlichnye vozrastnye periody" ("Neuro-Humoral Mechanisms for the Regulation of Homeostasis and Growth Intensity in Various Age Periods"). In the book Vedushchie faktory ontogeneza ("Major Factors of Ontogenesis"). Publ.: Naukova dumka, Kiev, 1972, 232 - 243.

9. Rozanova, V. D., Musin, B. S. "Razvitie dykhatel'noy i serdechno-sosudistoy sistem u kryv v ontogeneze v usloviyakh trenirovki (k mekhanizmu vozniknoveniya tonusa vagusa)" ("Development of Respiratory and Cardiovascular Systems in Rats in Ontogenesis under Active Conditions (toward the mechanism of development of tonus vagus)"). Fiziologicheskiy zhurnal SSSR, 1968, 52, 11, 1327 - 1333.

10. Rozanova, V. D., Savkiy, T. G., Khodorova, N. A. "Izmenenie energotrat i soderzhaniya katekholaminov v mozge i nadpocheknikakh u kryv v usloviyakh myshechnykh nagruzok i pri gipokinezii" ("Changes in Energy Expenditure and Catecholamine Content in the Brain and Adrenal Glands of Rats under Conditions of Muscle Load and under Hypokinesia"). Fiziologicheskiy zhurnal SSSR, 1976, 62, 2, 304 - 309.

11. Savkiv, T. G. K kharakteristike osobennostey energetiki organizma v zavisimosti ot pola pri razvitii v usloviyakh deystviya razlichnykh form skeletno-myshechnykh nagruzok ("Characteristic Features of Energy in the Organism as related to Sex during Development under Conditions of Various Forms of Skeletal Muscle Loads"). Dissertation, Moscow, 1974.
12. Ugolhaeva, I. S. "Osobennosti gomeostaticheskoy reaktsii pri gipoksicheskoy gipoksii u sobak v razlichnye voznrastnye periody" ("Features of Homeostatic Reaction During Hypoxic Hypoxia in Dogs at Various Age Periods"). Byulleten' eksperimental'noy biologii i meditsiny, 1964, 58, 9, 34 - 39.
13. Vlk I., Tucek, S. "Distribution of Acetylcholine in the Auricles of the Mammalian Heart. Physiol. bohemoslov., 1961, 10, 65 - 69.
14. Vlk I., Tucek, S. "Changes in the Acetylcholine content and Cholinesterase activity in dog atria during early postnatal period." Physiol. bohemoslov, 1962, 11, 53 - 57.

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